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Review Article

Role of interleukin-12 in AIDS pathogenesis

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Summary

Interleukin-12 (IL-12) is a disulfide-linked heterodimeric cytokine of 70 kDa formed by a heavy chain of 40 kDa (p40) and a light chain of 35 kDa (p35). Monocyte/macrophages are the physiologically most relevant producers of IL-12, in response to both Gram-positive and Gram-negative bacteria, bacterial products and intracellular parasites. Although IL-12 has an enhancing effect on the survival and growth of early hematopoietic progenitor cells, most of IL-12 biological activity has been described on T and natural killer (NK) cells, where it induces production of cytokines, primarily interferon (IFN)-γ, enhances cytotoxic activity and, in cooperation with other stimuli, increases proliferation. IL-12 induces development of T helper type 1 (Th1) cells and the equilibrium between IL-12 and IL-4 is probably important for the balance *in vivo* between Th1 and Th2 responses. IL-12 plays an important role in host resistance to infection, in particularly to intracellular pathogens, by activating macrophages through induction of IFN-γ from T and NK cells and by enhancing cell-mediated immune responses dependent on Th1 cell development. IL-12 is also an effective adjuvant in vaccines thus favoring the development of protective Th1 memory responses. The ability of peripheral blood mononuclear cells (PBMC) from HIV-seropositive individuals to produce IL-12 is impaired in response to bacterial stimulation, and IL-12 has been shown to restore some of these depressed immunological functions *in vitro*, suggesting that a defect in IL-12 production may have a pathogenic role in the immunodeficiency of HIV-infected individuals. Based on these observations, phase I trials in HIV patients have shown modest tolerable toxicity and phase II trials will be initiated soon.

Introduction

The response of the organism to infectious pathogens involves a complex interaction between immune and inflammatory cells whose function is largely regulated by a network of soluble mediators or cytokines. Cytokines play an important functional role during the interactions between effector cells and accessory regulatory cells during host defense mechanisms in response to pathogens in antigen-specific, adaptive immunity and in natural resistance. The critical role of cytokines in the regulation of the immune response and their multiple and often overlapping effects suggests that deficient or abnormal production may result in clinical immunodeficiency.

Interleukin-12 (IL-12), also called natural killer cell stimulatory factor or cytotoxic lymphocyte maturation factor, is a heterodimeric cytokine originally described as a factor which induces the production of interferon (IFN)-γ from T and NK cells, resulting in an enhanced cytotoxic activity of NK cells, comitogenic effects on resting T cells and synergistic activity with IL-2 in the induction of lymphokine activated killer (LAK) cells generation (1, 2).

Structure and receptors

Purified IL-12 appears in SDS-PAGE as a 70 kDa heterodimeric protein that resolves under reduced conditions to two chains of 35 and 40 kDa. N-terminal sequences of the p40, p35 and p70 heterodimer, as well as tryptic peptides sequences, have been determined and the cDNA for the two genes encoding p40 and p35 has been cloned (3, 4). The two chains have unrelated sequences and are

encoded by two distinct genes; the IL-12 p40 gene has been mapped to human chromosome 5q31-q33, a region encoding several cytokine receptors and cytokines, and the IL-12 p35 gene mapped to human chromosome 3p12-q13.2 (5). Co-transfection of cDNA of both genes is required for production of a biologically active IL-12 heterodimer, although no functions have yet been associated with the isolated p40 or p35 chain (3, 4). The p40 cDNA sequence encodes a 328 amino acid polypeptide with a 22 amino acid hydrophobic signal sequence which corresponds to a mature protein with a calculated Mr of 34,700 containing 10 cysteine residues, 4 *N*-linked glycosylation sites and 1 consensus heparin binding site. The p35 cDNA sequence encodes a 253 amino acid polypeptide with two potential initiation codons, one compatible with a secreted protein and the other possibly compatible with a membrane-expressed protein. The secreted form of p35 has a calculated Mr of 27,500 with 7 cysteine residues and 3 *N*-glycosylation sites. The primary amino acid sequence of the p35 chain indicates an alpha-helical rich sequence, similar to many other cytokines (3).

When the p35 sequence was compared with the most conserved regions of human and murine IL-6 and G-CSF, many amino acids that were conserved in the IL-6 and G-CSF, were also observed to be present in the IL-12/p35 sequence (3). Of interest, the p40 sequence was found not to be homologous with other cytokines, but to belong to the hematopoietin receptor family. When the p40 amino acid sequence was compared with the extracellular domain of IL-6 receptor, almost a 25% homology was observed with many conservative substitutions. Both the IL-6 receptor and p40 have an *N*-terminal immunoglobin-like domain and are closely related to G-CSF, suggesting that these three molecules represent a subgroup within the superfamily.

The receptor for IL-12 remains at present poorly characterized. Binding analysis of radiolabeled IL-12 indicated 10^3 to 10^4 binding sites with a K_d of 100-600 pM on activated but not resting human T and NK cells (6). Further analysis in activated T cells has identified a low number of IL-12 binding sites with a much higher affinity (K_d = 2-20 pM) (6). A cDNA encoding a component of the IL-12 receptor has been isolated which encodes a 662 amino acid transmembrane protein with homology to gp130, one of the chains of the IL-6 receptor and other cytokines (7). However, cells transfected with this cDNA bind IL-12 with an affinity of only 2-5 nM, suggesting that this molecule may be only one chain of a multichain receptor (7).

Production of IL-2

Although IL-12 was originally isolated from B cell lines, it is now known to be produced primarily by phagocytic cells (monocytes/macrophages and neutrophils) and by antigen-presenting cells (dendritic and skin Langerhans cells) (8-10). Resting B cells appear to be poor producers of IL-12 (8); however, most Epstein Barr virus (EBV)-transformed B cell lines and EBV-positive lymphomas produce IL-12 with the highest levels of production observed in EBV-positive cell lines derived from AIDS-associated lymphomas (11). The production of IL-12 by phagocytic cells can be induced by various bacteria, bacterial products, intracellular pathogens and viruses (8, 12).

Treatment with bacterial products such as heat-fixed *Staphylococcus aureus* Cowan 1 strain or lipopolysaccharide (LPS) induced both an excess of the p40 chain and up to 0.5 ng/ml of the biologically active p70 heterodimer by resting peripheral blood mononuclear cells (PBMC) (8). The physiological significance of the overproduction of the free p40 chain is not clear. Within PBMC, monocytes are responsible for most IL-12 production. Nonadherent peripheral blood lymphocytes (PBL), depleted of monocytes, can produce IL-12 in response to *S. aureus*; the nonadherent producer cells are B cells and other, as yet unidentified, MHC class II positive cells. Both *S. aureus* and LPS induced a many-fold but transient increase of p40 mRNA with a peak around 3 h after stimulation (8). Neither production of IL-12 nor accumulation of p40 mRNA was observed in purified T or NK cells, on which the presence of IL-12 receptors has been unequivocally demonstrated (8). As observed for many other cell types, hematopoietic or not, T and NK cells express p35 mRNA but not p40 mRNA, and the expression is highly regulated and present only in cell types able to produce the biologically active IL-12 heterodimer. The induction of IL-12 by LPS of Gram-negative bacteria has been well defined, but the components of Gram-positive bacteria and other pathogens that induce IL-12 remain uncharacterized (8). Cleveland *et al.* (13) have reported that lipoteichoic acid preparations from various Gram-positive bacteria induce IL-12 through a mechanism involving the CD14 receptor.

Recently, bacterial DNA was also shown to induce IL-12 production, and this activity has been found to be dependent on the presence of nonmethylated CpG repeats (14). Dendritic cells can be stimulated to produce IL-12 by bacterial stimulation (15); however, the interaction of the dendritic cells with antigen-activated T cells appears to be the primary mechanism of IL-12 production by this cell type (9, 15). The ability of activated T cells to induce IL-12 production in both macrophages and dendritic cells depends on the interaction of CD40 ligand expressed on T cells, cells with CD40 on macrophages or dendritic cells (16-18). Thus, in both systems, IL-12 production can be driven by a pathogen-induced T cell-independent mechanism and by a separate T cell-dependent one which depends on a CD40-CD40 ligand interaction. Other cells, such as mast cells, keratinocytes and neural cells have been reported to produce IL-12. The production of IL-12 *in vivo* and *in vitro* has now been associated with an increasing number of pathogens, including bacteria, intracellular protozoal, fungi and viruses. The production of IL-12 after infection is followed by and required for production of IFN-γ by NK and T cells, although other cofactors, including IL-1β and tumor necrosis factor

(TNF), are also necessary for optimal IFN-γ production (19). The IL-12-dependent production of IFN-γ results in activation of phagocytic cells, in a proinflammatory loop. IFN-γ activated phagocytic cells are also primed to produce much higher levels of IL-12 in a powerful positive feedback mechanism. Although IFN-γ is not an absolute requirement for IL-12 production, this feedback mechanism is particularly powerful and is probably required for optimal production of IL-12.

Because of the toxic and in some cases lethal effects of overproduction of IL-12 (*e.g.*, endotoxic shock), effective mechanisms exist to limit its production or the ability of T and NK cells to respond to it. At present, IL-10 is the best studied inhibitor of IL-12 production in both phagocytic and dendritic cells (17, 20). The slightly delayed expression of IL-10 as compared to that of IL-12 and other proinflammatory cytokines both *in vivo* and *in vitro*, makes it a very effective downregulator of the IL-12 response. Several reports have indicated that IL-12 itself can induce T cells to produce IL-10, suggesting a model whereby IL-12 may limit its own production (21-23) by induction of its own negative regulator, IL-10.

Biological activities

Although most of the biological activities of IL-12 have been described using T and NK cells (1, 2, 8, 9), IL-12 has been shown to synergize with other hematopoietic factors, particularly steel factor, enhancing survival and growth of early hematopoietic progenitor cells, including cells with myeloid-B lymphoid bipotential and lineage-committed precursor cells (24-29). IL-12 induces IFN-γ production from resting and activated NK and T cells (1, 8, 9). Among T cells, both T cells with α-β T cell receptor (TCR) ($CD4^+$ and $CD8^+$) and T cells with γ-δ TCR (30) are induced to produce IFN-γ. The production of IFN-γ by resting PBL in response to IL-12 and IL-2 requires the participation of nonadherent MHC class II positive accessory cells different from either monocytes or B cells (24). IL-20 also induces the production by T and NK cells of other cytokines, including GM-CSF, TNF and IL-8 (1, 24, 30-33).

IL-12 treatment of resting PBL or purified NK cells results in enhancement of the spontaneous cytotoxic activity of $CD16^+$, $CD56^+$, $CD3^-$ and $CD5^-$ NK cells against a variety of NK-sensitive and insensitive target cells within a few hours (1, 34-36). The mechanism by which IL-12 increases the cytotoxic activity of NK cells is not known at the present time, but appears to involve an increased number of granules (36) and increased expression of cell adhesion molecules (34, 37) and granule-associated proteins (*e.g.*, perforin and serine esterases) (38-40).

IL-12 and differentiation of Th1 responses

Several studies *in vitro* in humans and *in vitro* and *in vivo* in mouse have assigned a role to IL-12 as a potent inducer of T helper type 1 (Th1) cell responses, acting antagonistically with IL-4, a major promoter of Th2 responses (25, 26, 46-48). IL-12 added to PBL from atopic patients in the presence of allergen induced the generation of Th1-like $CD4^+$ clones displaying a Th1 phenotype (high IFN-γ and low IL-4). Conversely, neutralizing anti-IL-12 antibodies added to cultures of bacterial antigen-stimulated PBL induced the generation of clones with a Th2-like phenotype (46). These data indicated that IL-12 is an inducer of human Th1 cell generation and that endogenous IL-12 produced in bacterial antigen-stimulated PBL cultures is required for Th1 cell generation. The ability of IL-12 to induce Th1 cell generation from naive T cells stimulated with antigens was best demonstrated using mouse strains transgenic for TCR that are specific for either ovalbumin (49) or cytochrome C (50). $CD4^+$ cells from these animals differentiated into Th1 cells when IL-12 was added to cultures stimulated with specific antigens and antigen-presenting cells and into Th2 cells when IL-4 was added. The role of IL-12 in Th1 cell differentiation, identified in many *in vitro* and *in vivo* studies, is supported by the finding that IL-12 p40 knockout mice have severely depressed Th1 responses (51), as shown by the reduced ability of T cells to produce IFN-γ, although T cells producing normal levels of IL-2 are still generated. IL-12 acts *in vivo* and *in vitro* only during the first few days of T cell expansion or response to antigen; after that, the addition of exogenous IL-12 has little effect.

The question of whether IL-12 induces differentiation of uncommitted T cells to Th1 or selectively expands precommitted Th1 cells, was analyzed by using culture conditions which clonally expanded virtually every T cell from human PBL, and thus, excluded selection mechanisms. T cell cloned in the presence of IL-12, both $CD4^+$ and $CD8^+$, were all able to produce high amounts of IFN-γ, whereas T cell cloned in the presence of anti-IL-12 produced no or very low levels of IFN-γ; production of IL-4, however, was only minimally affected by the absence or presence of IL-12. These results indicate that IL-12 has a direct differentiating effect on all T cells, irreversibly priming them for a high IFN-γ response to subsequent stimulation.

IL-12 and infectious diseases

Emerging evidence points to an early role for IL-12 in mediating and controlling resistance to bacterial and intracellular parasitic infections, while exogenously added IL-12 has been demonstrated to have substantial antiviral activity. Thus, IL-12 plays a key role during host defense to infectious diseases (26, 52-54). The best experimental model to analyze the ability of IL-12 to favor Th1-like immune response is the infection of mice with the protozoan parasite *Leishmania major*. Unlike resistant mouse strains such as C3H which respond to *L. major* with a protective Th1 response, BALB/C mice develop a Th2 response which does not prevent progression of the infection. Continuous systemic treatment with IL-12 starting at the beginning of infection protects susceptible

BALB/C mice from *L. major* infection (25, 26, 54). The curative effect of IL-12 is paralleled by a shift from a Th2 to a Th1 type of response with early IFN-γ production and depression of IL-4 production (26, 54). Treatment with IL-12 initiated a few days after infection is inefficient, demonstrating that it is difficult to change an established Th2 response (26, 54). Immunization of BALB/C with soluble *L. major* extracts results in induction of a Th2 response that is not protective, but vaccination with soluble *L. major* in combination with IL-12 induces a memory Th1 response that completely protects BALB/C mice from subsequent infection with *L. major* (26, 54). This approach has been confirmed in *S. mansoni* infection, where a vaccine including IL-12 as an adjuvant prevents the Th2 response that causes pathologic granuloma formation (54). Recently, Hoffman *et al.* (55) described the *in vivo* protective effect of IL-12 administration against malaria in monkeys.

IL-12 and AIDS

Infection with HIV is associated with a wide spectrum of clinical stages ranging from asymptomatic illness to full-blown AIDS. A variety of profound abnormalities have been described not only in patients with AIDS-related complex and AIDS, but also in asymptomatic subjects (56). Infection with HIV is associated with a gradual loss of immunological function that results ultimately in numerous opportunistic diseases. It is increasingly evident that progression of the disease is closely associated with abnormal functioning of the immune system. HIV infection affects T, B and monocyte functions even before $CD4^+$ T cell depletion occurs. Besides a general decrease in the number of $CD4^+$ T cells, HIV-infected patients, early in the course of infection, have an imbalance in the function and activation of T helper cells (57, 58).

Although a tremendous amount of progress has been achieved in HIV research during the last decade, the pathogenesis of AIDS is still unclear. The nature and potential role of the cell-mediated and humoral immune responses against HIV have not yet been well characterized. Although animal models have demonstrated that protective immunity can be induced to other retroviruses, it remains to be established whether immune responses against HIV may prevent HIV primary infection and/or protect against disease progression in infected individuals. Knowing which immune responses to HIV may be protective is critical for the rational design of an effective vaccine.

Efforts are currently under way to develop new effective immunomodulatory agents which, in association with antiretroviral drugs, may be of beneficial therapeutic use. A great deal of information on the role of several cytokines during HIV infection has been accumulated, although the relative extent of their expression remains unclear. An *in vitro* imbalance in the Th1/Th2 cytokines with a possible shift from a Th1 to a Th2 response has been proposed by Clerici and Shearer (59) to be critical in the etiology of HIV infection by contributing to the immune dysregulation associated with HIV infection. The same authors proposed that resistance to HIV infection *versus* progression to AIDS is dependent on an efficient cellular immunity and therefore a dominance of Th1 over Th2 responses.

PBMC from HIV-infected patients produced 10- to 20-fold less IL-12 free p40 chain and 5-fold less biologically active p70 heterodimer when challenged *in vitro* with the common human pathogen *S. aureus* (60). In contrast, PBMC from HIV-infected individuals and uninfected control donors produced very similar levels of TNF-α, IL-1β and IL-10, and PBMC from HIV-infected patients produced 3- to 4-fold more IL-6 compared uninfected control donors (60). The decreased production of IL-12 p70 heterodimer has been confirmed by several other investigators (60-65). The specific deficiency in the production of IL-12, while other inflammatory cytokines are produced normally or at increased levels by HIV-infected patients, suggests a possible role of IL-12 deficiency in the pathogenesis of HIV. Similar results were obtained with enriched preparations of monocytes from HIV-infected patients, and HIV infection of monocyte cultures *in vitro* induced a marked reduction of their ability to produce IL-12 (60). However, in both the peripheral blood of patients and in infected monocyte cultures, only a small proportion of monocytes is expected to be directly infected by the virus, indicating that exposure to the virus, virus products or virus-induced cellular products rather than direct infection of the IL-12 producer cells might be responsible for the inhibition of IL-12 production.

Within PBMC, IL-12 producer cells are known to be represented not only by monocytes but also by other HLA class II positive cells and, in part, by lymphocytes. Because of mounting evidence that dendritic cells might be powerful producers of IL-12, it is tempting to postulate that the class II positive IL-12 producing cells are dendritic cells. The deficiency in the antigen-presenting activity of dendritic cells from HIV-infected patients described in some studies but not in others (66, 67) might be consistent with their reduced ability to produce IL-12.

The mechanism by which exposure to HIV reduces the ability of PBMC to produce IL-12 remains elusive. HIV-infected patients, at least in some phases of their disease, have been described to overproduce certain Th2 type cytokines, in particular IL-4 and IL-10. Because Th2 cytokines, particularly IL-10, are able to suppress IL-12 production (25, 26, 54, 68), it could be hypothesized that exposure of PBMC *in vivo* or *in vitro* to these cytokines is responsible for decreased IL-12 production. However, these cytokines have a suppressive effect on the production of IL-12 and all inflammatory cytokines, making it difficult to explain the selective IL-12 deficiency (60, 65). In the short-term *S. aureus*-stimulated culture of PBMC, production of IL-10 but not IL-4 was observed (60, 64, 65, 68). Furthermore, when PBMC from controls or patients are exposed to IL-4 or IL-13 for an extended period of time, their ability to produce IL-12 is boosted rather

than inhibited (69, 70), casting doubt on a potential role for IL-4 or IL-13 in promoting selective IL-12 deficiency.

The role of IL-10, the prototypical antiinflammatory cytokine, in determining the inability of patient PBMC to produce IL-12 is a controversial one. Chougnet *et al.* (64, 71) reported that IL-10 production is increased in cultures of cells from HIV^+ patients compared to those from healthy controls, and this hyperproduction may explain the impairment in IL-12 production seen during HIV disease. However, we and others have failed to observe significant overproduction of IL-10 by PBMC from a cohort of >100 HIV^+ patients (60, 65, 68, 72, 73). In addition, it is unlikely that the inhibitory effect of endogenously produced IL-10 was selectively exerted on IL-12 production alone with normal or increased levels of IL-1β, TNF-α and IL-6 levels (60), since IL-10 has been demonstrated to directly inhibit the *in vitro* production of those monokines as well. Because endogenous IL-10 in culture is known to limit IL-12 production (20), the ability of neutralizing anti-IL-10 to correct the deficient IL-12 production from HIV patients was tested (60, 71). A similar increase in IL-12 production in the presence of the antibodies was observed in both HIV-infected patients and healthy controls, suggesting that IL-10 was most likely not responsible for the difference in production of IL-12 of PBMC preparations from patients and controls.

Recently, it was reported that prostaglandin E_2 (PGE_2) is a potent and selective inhibitor of IL-12 production (74). Because HIV infection of monocytes enhances their production of PGE_2 (75), and increased levels of cAMP in PBMC from patients have been reported (76), the possibility that PGE_2 plays a role in the deficient production of IL-12 has been investigated. However, consistent with the recent observation by Meyaard *et al.* (65), we detected only a minor elevation of *S. aureus*-induced PGE_2 production from HIV^+ PBMC as compared to control cells (70). Additionally, indomethacin, used at doses capable of inhibiting PGE_2 production, failed to increase or normalize IL-12 production in control and patient cells, respectively. Taken together, these results strongly suggest that PGE_2 is not primarily responsible for the depressed IL-12 levels observed in HIV^+ monocytes. A restoration of some T cell functions during HIV infection has been observed *in vitro* when intracellular cAMP levels were decreased (77). However, we find that adenosine analogs such as ddAdo and 3'AMP, known to reduce intracellular cAMP levels, failed to restore or enhance IL-12 production, strongly suggesting that decreased IL-12 production observed during HIV infection is independent of the high cAMP levels previously reported (77).

Another aspect of IL-12 production possibly involved in the deficient production in HIV-infected patients which merits investigation is the role that signaling by T cells plays in IL-12 induction. Evidence suggests that at least during antigen-specific stimulation, T cells are needed for induction of IL-12 production in antigen-presenting cells. Thus, it is conceivable that the observed IL-12 deficient production in the patients may in part depend on deficient signaling by activated T cells to the antigen-presenting cells. Because of the role of IL-12 in T cell activation, this mechanism of immunodeficiency would be self-amplifying. The deficient production of IL-12 was similarly observed in patients at various stages of disease progression, from asymptomatic patients with > 500 $CD4^+$ T cells/mm^3 to advanced AIDS patients with < 200 $CD4^+$ T cells/mm^3. Thus, the deficiency in IL-12 production represents a very early defect in HIV disease progression.

Because IL-12 has an important and likely key role in the initiation of cellular immunity, it is of interest that its deficient production appears to coincide with the early deficient proliferative response of patient T cells to recall antigens. There is evidence that in the absence of IL-12 production, the ability of antigen-presenting cells to induce a Th1 response and delayed-type hypersensitivity is reduced or eliminated. IL-12 has also been shown to be necessary for optimal proliferation and cytokine production in differentiated Th1 cells. Therefore, it is conceivable that, if in HIV-infected patients a generalized inability to produce IL-12 in response to infectious stimuli is present, ineffective cell-mediated and Th1 response or nonprotective Th2 responses might be generated upon infection and possibly even antigen tolerance or deletion of antigen-specific clones might be induced, contributing to the immunodeficient status of the patients.

Although *in vitro* T cell response and the ability of PBMC to respond to stimulation with IL-12 production are deficient, analysis of expression of cytokine mRNA in peripheral blood or lymph nodes of HIV patients showed increased expression of Th1 cytokines, including mRNA for IFN-γ, a cytokine dependent on IL-12 for optimal induction. Although these observations may question the hypothesis of a predominant Th2 response in HIV-infected patients, they are not incompatible with the possible deficiency of IL-12 production in response to infection. Because of infections and other immunological stimulations, an activation state in patient lymphocytes and monocyte/macrophages has been described. In these conditions, constitutive expression of mRNA for activated lymphocyte products, including IFN-γ, and of proinflammatory cytokines in macrophages and antigen-presenting cells, including IL-12, is to be expected.

Although HIV-infected patients appear to be deficient in their ability to produce IL-12, their T and NK cells *in vitro* have been shown to respond normally to IL-12. When IL-12 is produced in response to an infection, the major effects of IL-12, regulating inflammation and immune response, take place at three major stages: first, NK cells are activated and IFN-γ production is induced in both T and NK cells; second, differentiation of Th1 cells is induced, depending on an irreversible priming or high IFN-γ production and inhibition of Th2 responses; third, IL-12 is required for optimal proliferation and cytokine production. IL-12 has been shown to be active at all three stages on T and NK cells from HIV-infected patients.

Patients with advanced HIV disease often exhibit very reduced cytotoxic activity in their peripheral blood NK cells, although the number of NK cells is not decreased. IL-12 treatment *in vitro* enhances the NK-mediated

cytotoxic activity of PBL from HIV-infected patients in a manner similar to the effect observed in PBL from healthy donors (35, 37). This NK-enhancing effect of IL-12 is particularly evident on lymphocytes from advanced patients with almost no NK cytotoxic activity in whom IL-12 restored the cytotoxic activity to levels close to those of healthy donors (35). IL-12 enhanced the NK cytotoxic activity of patient lymphocytes against both tumor target cells and virus-infected target cells (35); interestingly, IL-12 can also boost the NK cytotoxic activity of lymphocytes from healthy donors against HIV-infected target cells (35, 36). On freshly purified PBL from HIV-infected patients, IL-12 alone or in synergy with IL-2 induced IFN-γ production, although in advanced patients the levels were somewhat lower than those observed in healthy donors (35). IL-12 also enhanced phytohemagglutinin (PHA)-induced IFN-γ production in lymphocytes from HIV-infected patients, and almost completely corrected the low production in some patients (35). The reduced ability of patient lymphocytes to produce IFN-γ upon *in vitro* stimulation is not necessarily in contrast to the finding of constitutive expression of IFN-γ mRNA in peripheral blood and lymph nodes of patients, and both probably reflect the complex alterations of immune reactivity in HIV disease (78, 79).

The induction of IFN-γ production and the enhancement of NK cytotoxicity represent rapid and short-lasting effects of IL-12, which may not easily lend themselves to a permanent *in vivo* therapeutic effect. Therefore, it was important to demonstrate whether IL-12 could prime T cell clones from HIV-infected patients for high IFN-γ production, an effect that was shown to be long-lasting and possibly irreversible. Peripheral blood T cells from 10 HIV-infected patients at different stages of disease were cloned by limiting dilution in the presence or absence of IL-12 for the first 2 weeks of culture (80). Very high efficiency of clonal expansion was obtained by culture in the presence of irradiated feeder cells, PHA and IL-2. On average, $CD4^+$ clones cultured in the presence of IL-2 produced 10-fold more and $CD8^+$ clones 5-fold more IFN-γ than clones originated in the absence of IL-12 when restimulated after 1 month expansion by anti-CD3 and phorbol diester (80). This priming effect, which is analogous to that observed with T cells from healthy donors, was observed with patients at any stage of the disease (80); interestingly, IL-12 induced a very significant priming for high IFN-γ production even in $CD8^+$ T cells from patients with almost no $CD4^+$ cells, and from whose T cells no $CD4^+$ clones were obtained (80). This priming for IFN-γ production is possibly the most important direct effect by which IL-12 might be an effective Th1 inducer even in HIV-infected patients. An insufficient IL-12 induced priming of patient $CD4^+$ T cells *in vivo* might possibly be responsible for the significantly lower ability to produce IFN-γ observed with patient $CD4^+$ clones generated in the absence of IL-12 (68, 80).

IL-12 was also shown to enhance the depressed proliferation of HIV patients to recall antigens, including HIV peptide, influenza virus, PHA and alloantigens, to levels close to those of healthy donors (81-85). These results are consistent with the ability of IL-12 to enhance the proliferative response to antigens, alloantigens and mitogens observed with T cells from healthy individuals (1, 2, 30) and to the primary and, in some cases, obligatory role of IL-12 in antigen-induced proliferation of memory T cells and differentiated Th1 cells. The *in vitro* enhancing effect of IL-12 on HIV patient T cell proliferation could be due to activation of unresponsive T cells or to replacement of insufficient IL-12 produced *in vitro* by patient antigen-presenting cells.

Another important function of IL-12 shared with other Th1 cytokines such as IL-2, is its ability to prevent mitogen- or anti-CD3-induced programmed cell death in T cells from HIV^+ donors (86-88). IL-12 also inhibits apoptosis induced by gp120 or CD4 cross-linking and CD3/TCR activation in a human Th1 clone (86), an *in vitro* mechanism of induction of apoptosis which may mimic one of the pathogenic processes in HIV infection. Because death by apoptosis is one of the mechanisms proposed for $CD4^+$ cell depletion in AIDS and could be favored by reduced production of Th1 cytokines including IL-12, the ability of IL-12 to prevent T cell receptor-induced apoptosis in patient T cells represents a potentially important therapeutic function of this cytokine.

Conclusions

The possible defective ability of HIV-infected patients to produce IL-12, the apparently intact responsiveness of patient T cells to IL-12 and the ability of IL-12 to enhance some of the depressed immune functions of patients *in vitro*, make this cytokine a candidate for HIV disease therapy. The *in vitro* results suggest that IL-12 may be effective in HIV-infected patients in inducing a protective Th1 response against intracellular bacteria and parasites and as an adjuvant prophylactic vaccine against these pathogens. IL-12 has proven to be extremely effective therapeutically and prophylactically in experimental animals and several of the pathogens tested (*e.g.*, *T. gondii* and *M. avium*) represent common opportunistic infections in AIDS patients. The future understanding of the molecular nature of the inhibition of IL-12 production and of its timing during infection will help in evaluating a possible role of IL-12 in the pathogenesis of HIV infection.

Taken together, the *in vitro* results have given rise to the question of whether a possible therapeutic use of IL-12 in AIDS patients is feasible, as a boosting agent of the cellular immune response against opportunistic pathogens or possibly against HIV itself, and combined as an adjuvant in therapeutic or preventative vaccination against HIV and other pathogens. The greatest uncertainty remains, however, as to how IL-12, a cytokine with a powerful effect on lymphokine production, might alter the balance of cytokines affecting HIV replication, and clinically, whether a possible potentiation of cell-mediated immunity would be associated with a concomitant antiviral effect or with an enhancement of HIV replication and

the mechanisms responsible for $CD4^+$ T cell depletion. The concern that IL-12, through activating $CD4^+$, may increase HIV replication *in vivo* is mitigated by the observation that Th1 clones appear to be less permissive than Th2 clones for HIV replication (72, 73), thus raising the possibility that IL-12 *in vivo* might decrease rather than increase virus replication.

All of these factors obviously need to be considered in the careful planning and evaluation of clinical trials. It is evident that the treatment of a syndrome with multiple immunological anomalies such as AIDS would be facilitated by a combined use of multiple immune regulators and antiviral agents that would offer the possibility to correct and/or compensate for some of the immunological dysfunction while exerting a direct antiviral effect.

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